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14. ABSTRACT
We have initiated our search for anion receptors using the hypothesis that the binding of complex anions (e.g. ATP, ADP, nitrotyrosine, phosphotyrosine, etc for these model studies), will require a domain for the binding of the more hydrophobic portion of the molecule (the nucleobase) and a domain for desolvating and subsequently binding the phosphate portion of the anion. To achieve this end we have designed monomers that will form a cavity to bind the organic portion of the organophosphates selectively while also providing electrostatic interactions to stabilize the phosphate anion. Disulfide exchange was selected as the reversible reaction to generate the library, as it is

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Michel Gagne
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Report Title

UNC Center for Dynamic Combinatorial Chemistry

ABSTRACT

We have initiated our search for anion receptors using the hypothesis that the binding of complex anions (e.g. ATP, ADP, nitrotyrosine, phosphotyrosine, etc for these model studies), will require a domain for the binding of the more hydrophobic portion of the molecule (the nucleobase) and a domain for desolvating and subsequently binding the phosphate portion of the anion. To achieve this end we have designed monomers that will form a cavity to bind the organic portion of the organophosphates selectively while also providing electrostatic interactions to stabilize the phosphate anion. Disulfide exchange was selected as the reversible reaction to generate the library, as it is compatible with aqueous solution and is well established for DCC. In addition, we have developed thioester exchange as an alternative reversible reaction because it is significantly faster than disulfide exchange.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

1) "Dynamic combinatorial libraries for the developments of the synthetic anion binding receptors", Oct. 12, 2009, DCC intergroup meeting (S. M. Venkata/Gagné/Waters).
2) "Dynamic Cyclic Thiodepsipeptide Libraries", Nov. 9, 2009, DCC intergroup meeting (S. Ghosh/Gagné/Waters).
3) "Dynamic combinatorial libraries for the developments of the synthetic anion binding receptors - update", Feb. 8, 2010, DCC intergroup meeting (S. M. Venkata/Gagné/Waters).
4) "Dynamic Cyclic Thiodepsipeptide Libraries-update", Mar. 8, 2010, DCC intergroup meeting (S. Ghosh/Gagné/Waters).
5) "Developments of the synthetic receptors for anions",Mar. 19, 2010, group meeting (S. M. Venkata/ Gagné/Waters).
6) "Dynamic Cyclic Thiodepsipeptide Libraries-update", June 8, 2010, group meeting (S. Ghosh/Gagné/Waters).
7) "Developments of the synthetic receptors for anions - update", June 21, 2010, group meeting (S. M. Venkata/ Gagné/Waters).
8) "Developmen of DCC assay for protein-protein interaction inhibitors", Aug. 8, 2010, group meeting (S. Ghosh/Gagné/Waters).
9) "Developing small molecules for anion binding using dynamic combinatorial chemistry", Oct. 26, 2010, group meeting (J. Beaver/Waters).
10) "Synthesis of building blocks for inhibition of p53-HDM2 interaction and binding tri-methyl lysine on histone tail using dynamic combinatorial chemistry", Dec. 3, 2010, DCC intergroup meeting (S. Ghosh/Gagné/Waters).
11) "Developing small molecules for anion binding using dynamic combinatorial chemistry-update", Jan. 10, 2011, group meeting (J. Beaver/Waters).
12) "Developmen of DCC assay for protein-protein interaction inhibitors-update", Feb. 14, 2011, group meeting (S. Ghosh/Gagné/Waters).
13) "Development various peptides for G- Quadruplex DNA using dynamic combinatorial chemistry", Mar. 14, 2011, DCC intergroup meeting (E. Cline/Gagné/Waters).
14) "Developing small molecules for post translational modifications using dynamic combinatorial chemistry-update", Aug. 4, 2011, DCC intergroup meeting (J. Beaver/Gagné/Waters).
15) "Development various peptides for G- Quadruplex DNA using dynamic combinatorial chemistry-update", Oct. 18, 2011, DCC intergroup meeting (E. Cline/Gagné/Waters).
16) "Developing small molecules for anion binding using dynamic combinatorial chemistry-update", Feb. 2, 2012, DCC intergroup meeting (J. Beaver/Gagné/Waters).

17) "Development various peptides for anion binding using dynamic combinatorial chemistry", May 23, 2012, DCC intergroup meeting (E. Cline/Gagné/Waters).
18) "Developing small molecules for post translational modifications using dynamic combinatorial chemistry-update", Aug. 9, 2012, DCC intergroup meeting (J. Beaver/Gagné/Waters).
19) "Development of selective small molecules for post translational modifications using dynamic combinatorial chemistry-update", Sept. 14, 2012, group meeting (J. Beaver/Waters).
20) "The design od small molecue sensors for modified amino acids in water", Oct. 18, 2012, DCC intergroup meeting (J. Beaver/Gagné/Waters).
21) "Development various peptides for anion binding using dynamic combinatorial chemistry-update", Feb 14, 2013, DCC intergroup meeting (E. Cline/Gagné/Waters).
22) "Synthesis of a selective small molecule receptor for aRMe2", April 5, 2013, group meeting (J. Beaver/Waters).
23) "Synthesis of a selective small molecule receptor for aRMe2-update", June 14, 2013, group meeting (J. Beaver/Waters).
24) "A synthetic receptor for asymmetric dimethyl arginine" Joshua E. Beaver, Lindsey I. James, Natalie W. Rice, Marcey L. Waters, July 7-11, 2013, Poster presentation, 8th International Symposium on Macrocyclic and Supramolecular Chemistry (ISMSC-8), Arlington, Virginia, U.S.A.

Number of Presentations: 24.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received		Paper
04/09/2014	5.00	. A Synthetic Receptor for Asymmetric Dimethyl Arginine, ( )
04/09/2014	3.00	Soumyadip Ghosh, Lindsey A. Ingerman, Aaron G. Frye, Stephen J. Lee, Michel R. Gagne?, Marcey L. Waters. Dynamic Cyclic Thiodepsipeptide Libraries From Thiol-Thioester Exchange, Organic Letters (04 2010)
07/09/2008	1.00	Chung, Schiltz, Lee, and Gagne. The Effect of Gas-Phase Reactions on the Quantitation of Cyclic Hydrazone Libraries by Electrospray Ionization (ESI) Mass Spec, ( )
07/09/2008	2.00	chung, Hebling, Jorgenson, Severin, Lee, Gagné. “Deracemization of a Dynamic Combinatorial Library Induced by (–)-Cytidine and (–)-2-Thiocytidine”, ( )
TOTAL:	**4**	

Number of Manuscripts:

Books

Received	Paper

TOTAL:

Patents Submitted

Patents Awarded

Awards

Michel Gagne, AAAS Fellow
Michel Gagne, Mary Ann Smith Distinguished Professor

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Josh Beaver	0.25	
Elizabeth Cline	1.00	
FTE Equivalent:	**1.25**	
Total Number:	**2**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Dr. Soumyadip Ghosh	1.00
Dr. Srirama Venkata	1.00
FTE Equivalent:	**2.00**
Total Number:	**2**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Michel Gagne	0.08	
Marcey Waters	0.08	
FTE Equivalent:	**0.16**	
Total Number:	**2**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	Discipline
Julianne Bain	0.05	chemistry
Adam Hill	0.05	chemistry
FTE Equivalent:	**0.10**	
Total Number:	**2**	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	2.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:	2.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:	2.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	2.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	1.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME
Elizabeth Cline
Total Number: **1**

Names of other research staff

NAME PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

see attachment

Technology Transfer

FINAL REPORT

August 1, 2009- August 15, 2013

Decon Enabling Sciences

Submitted by: Marcey Waters and Michel Gagné

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Telephone: (919) 962-6341
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Fort Belvoir, Virginia 22060-6201
Telephone: (703) 767-XXXX
Email: [S&T Manager or relevant email address]

April 8, 2014

Project Number: BA09PHMXXX [edit as appropriate]
Contract Number: [If applicable]
Capability Area: Air Purification [edit as appropriate]
Period of Performance: Oct 01, 2009 – Aug 15, 2013

1. **PROJECT AND REPORT OVERVIEW [Note: this report, in its entirety, is intended to be significantly detailed, to facilitate collating the quarterly data into a comprehensive, thorough annual report.]**

   a. The objective of this project is to discover receptors able to tightly bind anions in complex environments using dynamic combinatorial chemistry (DCC), which is a high throughput method for identifying receptors from a library of compounds.

   b. We have initiated our search for anion receptors using the hypothesis that the binding of complex anions (e.g. ATP, ADP, nitrotyrosine, phosphotyrosine, etc for these model studies), will require a domain for the binding of the more hydrophobic portion of the molecule (the nucleobase) and a domain for desolvating and subsequently binding the phosphate portion of the anion. To achieve this end we have designed monomers that will form a cavity to bind the organic portion of the organophosphates selectively while also providing electrostatic interactions to stabilize the phosphate anion. Disulfide exchange was selected as the reversible reaction to generate the library, as it is compatible with aqueous solution and is well established for DCC. In addition, we have developed thioester exchange as an alternative reversible reaction because it is significantly faster than disulfide exchange.

   c. Key achievements

      1. Expanded the set of water-soluble well-behaved dithiol monomers to monomers containing amine and urea groups for DCC using disulfide exchange as the reversible reaction.

      2. Identified a synthetic receptor for the hydrophobic component of nitrotyrosine as a model anionic compound from a DCC library of dithiols.

      3. Studied the binding mode between the target guest and the identified receptors by NMR studies.

      4. Resynthesized the identified synthetic receptor to separate the conformational isomers.

      5. Determined the binding constants between the target compounds and the identified synthetic receptors using anisotropy titration method and Isothermal Titration Calorimetry (ITC) analysis.

      6. Synthesis thiol/thioester monomers for thioester exchange.

      7. Evaluate kinetics of thioester exchange and its structure-dependence.

      8. Screen for anion binding with thiol/thioester libraries.

      9. Development of DCL analysis methods using size exclusion chromatography

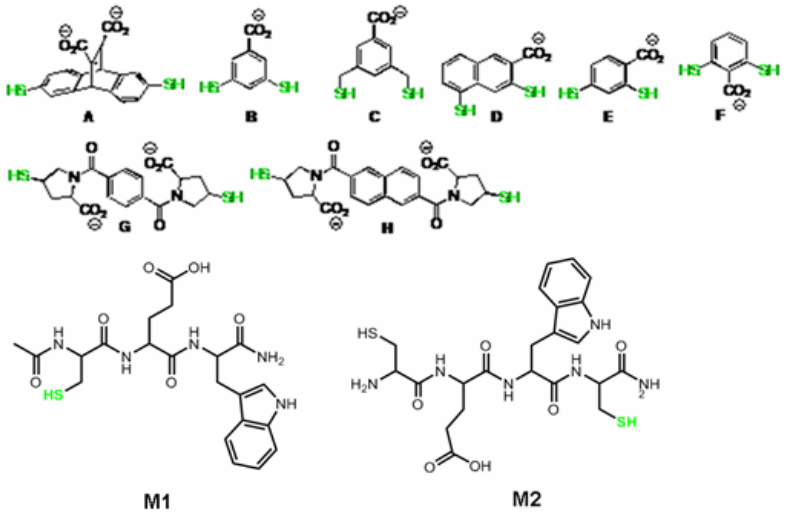
2. **TECHNICAL PROGRESS DURING THE REPORTING PERIOD** [This section will constitute the bulk of the report]

   a. <u>Task 1: Increase diversity of ditihiol monomers.</u>

      We have synthesized a set of dithiol monomers and a new set of monothiol monomers composed of four different amino acids to increase variability in the hydrophobic binding pocket (Figure 1a). We have synthesized a set of dithiol monomers having positively charged amine functions to increase variability in the hydrophobic binding pocket (Figure 1b). These monomers are positively charged and expected to show the stronger binding

affinity to anionic guests. The synthesis of another sets of new monomers has been initiated (Figure 1c). These monomers were the above monomer derivatives incorporating both amine groups and urea functions. In addition, the synthesis of binaphthyl-based monomers were initiated (Figure 1d). Additionally, The synthesis of binaphthyl-based monomers containing the positively charged amine groups were pursued (Figure 1e).

(a)



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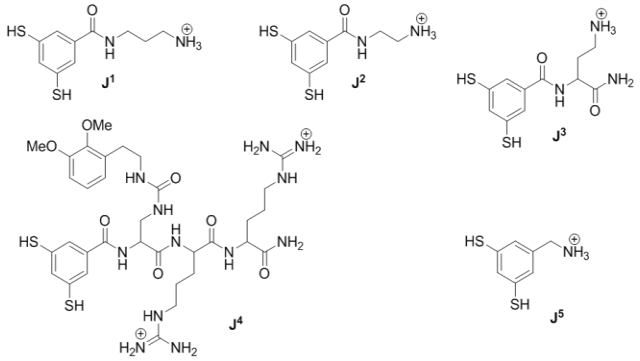
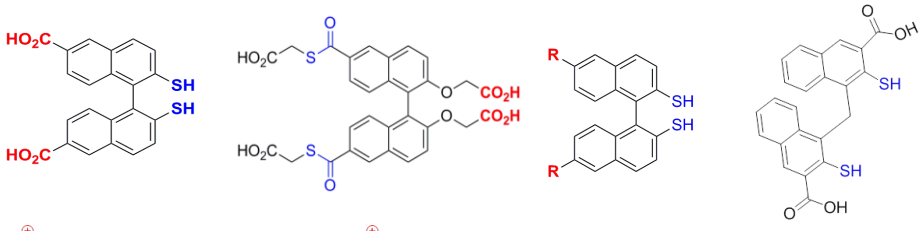


(c)

(d)



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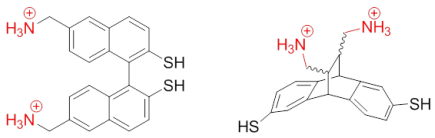


Figure 1. (a) The developed dithiol and monothiol monomers. (b) dithiol monomers containing the positively charged amine functions (c) dithiol monomer precursors containing amine functions and urea groups (d) binaphthyl-based dithiol monomers (e) binaphthyl-based dithiol monomers containing the positively charged amine functions.

b. Task 2.1: Screening vaious libraries composed of dithiol monomers and monothiol monmers against anionic target molecules, including ATP, ADP, AMP, GTP, phosphotyrosine, nitrotyrosine and tyrosine based peptides. We have screened of the developed dithiol monomers using several anionic target molecules.

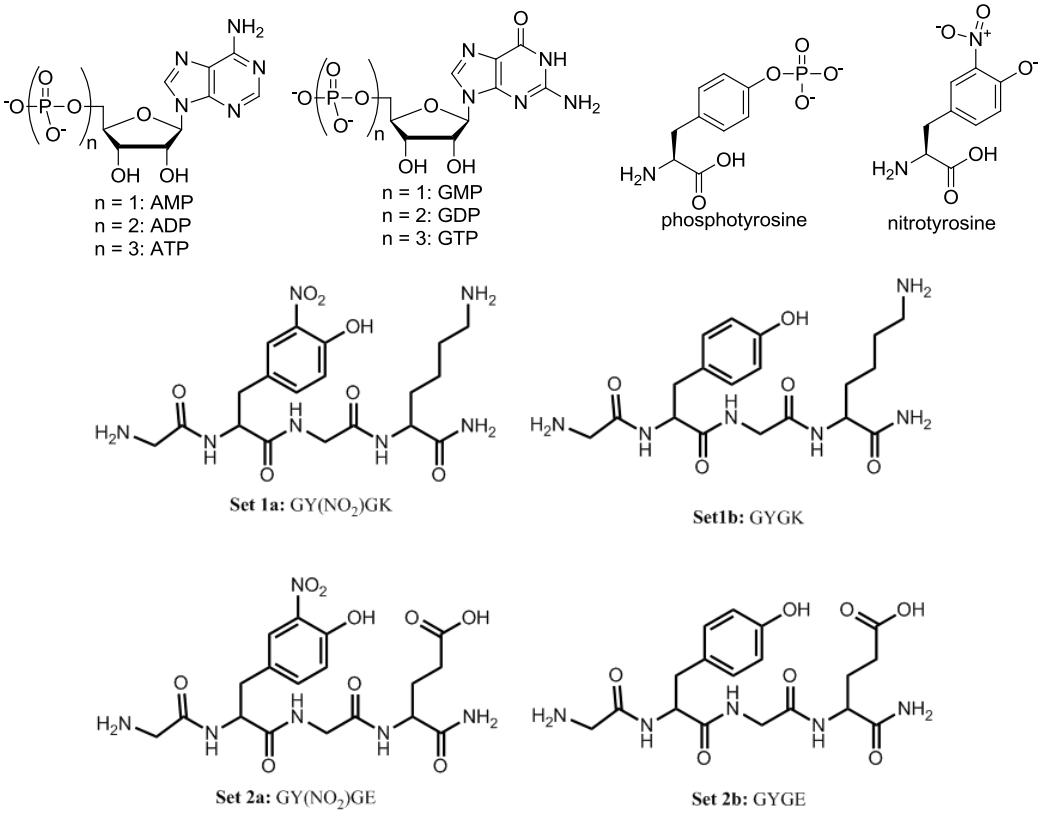
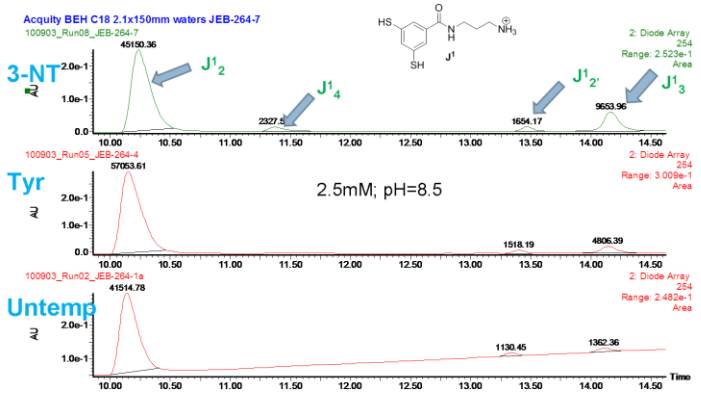


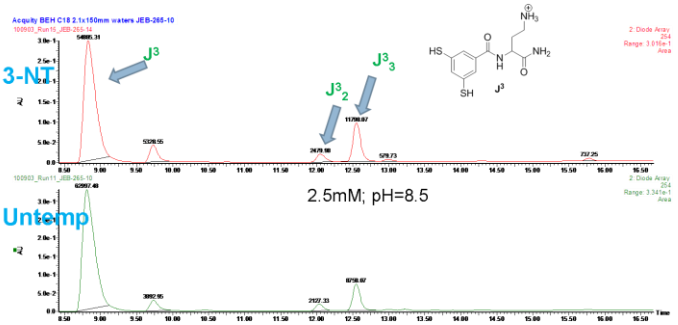
Figure 2.1. Anionic target molecules including nucleotides and tyrosine and nitrotyrosine peptides.

c. Task 2.2: Identification of receptors discovered in Task 2.1. In the screening of libraries composed of mixed dithiol monomers using tyrosine and nitrotyrosine peptide-based guests, the libraries first generated from two dithiol monomers. In the homo libraries generated from two dithiol monomers (J_1 and J_3 in Figure 1b), cyclic trimers were amplified by the nitrotyrosine peptide-based guest. In the hetero libraries formed from two dithiol monomers (J_1 and J_3 in Figure 1b), homo cyclic trimers were preliminary resulted by the nitrotyrosine peptide-based guest. The dithiol monomer containing the positively charged amine group has also been evaluated. With the nitrotyrosine peptide-based guest ($GY(NO_2)GK$), a cyclic tetramer was amplified (Figure 2.2d). In another homo libraries generated from the monomer A (Figure 1a), the cyclic trimer amplification by the nitrotyrosine peptide-based guest was confirmed (Figure 2.2e).

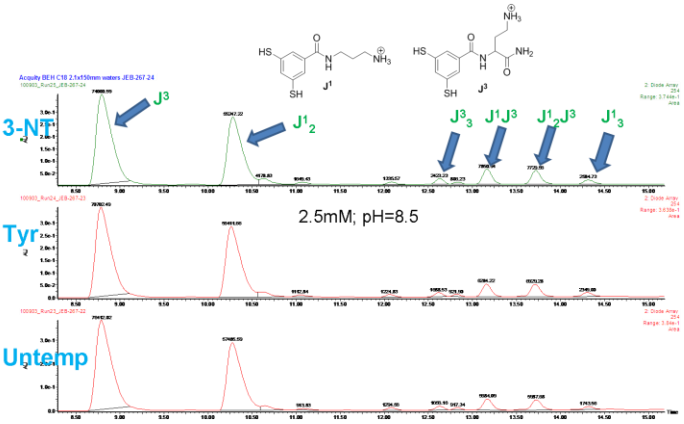
(a)


Acquity BEH C18 2.1x150mm waters JEB-264-7
3-NT
Tyr
Untemp
2.5mM; pH=8.5
Time

(b)


3-NT
Untemp
2.5mM; pH=8.5

(c)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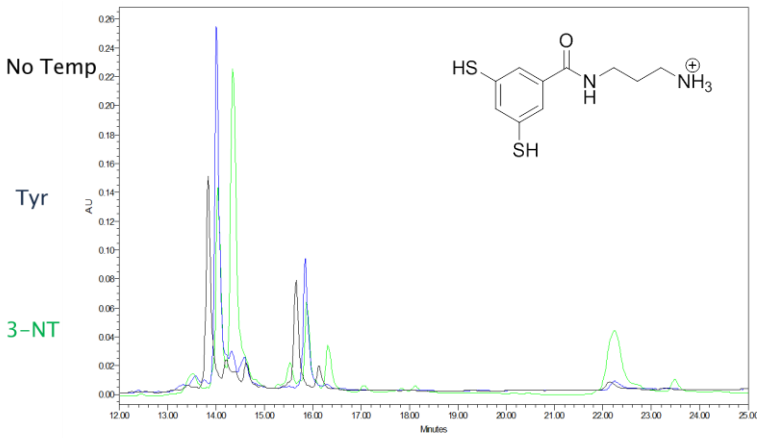
Tyr
Untemp
2.5mM; pH=8.5

(d)


No Temp
Tyr
3-NT
AU
Minutes

(e)

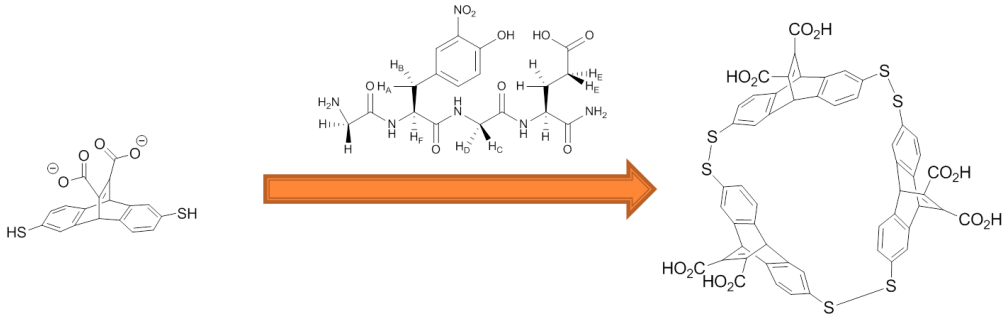
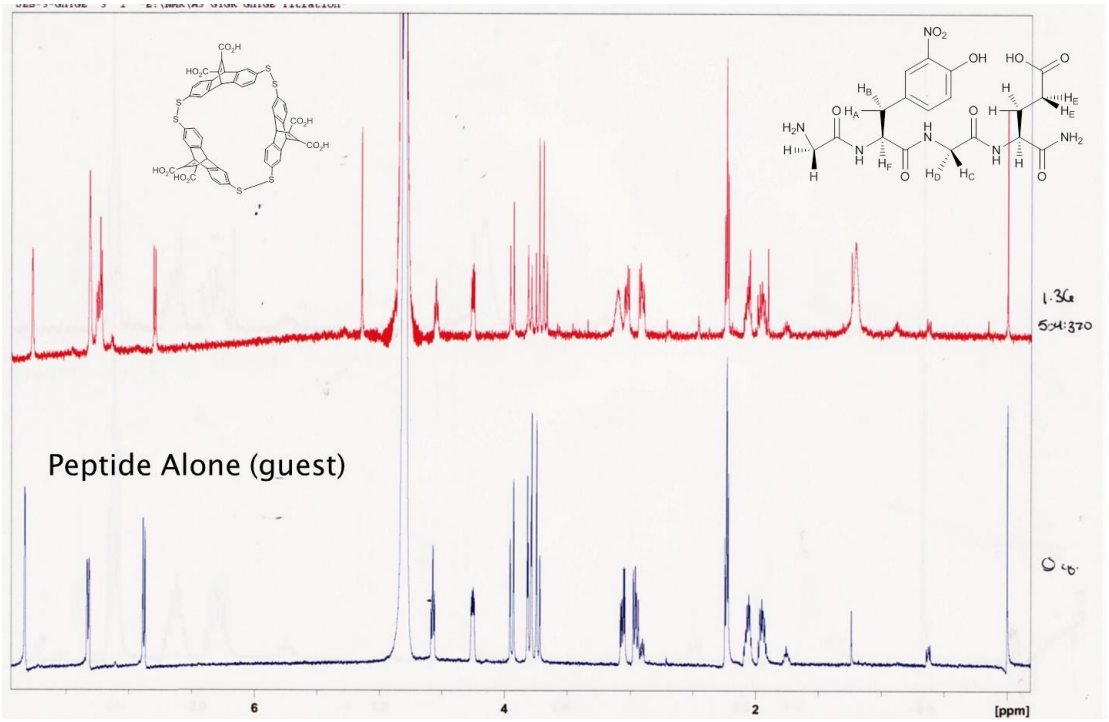
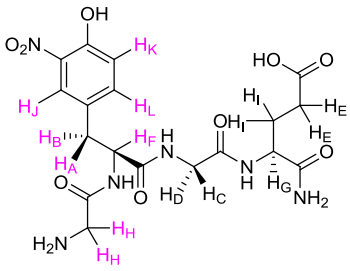


Figure 2.2. UPLC-UV trace (254 nm) of untemplated and guest-templated homo DCLs generated from (a) J^1 monomers and (b) J_3 monomers , and (c) UPLC-UV trace (254 nm) of untemplated and guest-templated hetero DCLs generated from J^1 monomers and J_3 monomers (guest: nitrotyrosine based peptide(3-NT) and a tyrosine based peptide (Try)). (d) UPLC-UV trace (254 nm) of untemplated and guest-templated homo DCLs generated from the monomer A containing the positively charged amine group (guest: a nitrotyrosine based peptide (3-NT) and a tyrosine based peptide (Try)) (e) The identified cyclic homo trimers amplified by the nitrotyrosine peptide-based guest.

d. <u>Task 3: Study of the binding mode between the target guest and the identified receptors by NMR studies.</u> To find the binding mode, the behaviors of the guest with or without the receptors in solution were monitored by NMR spectra (Figure 3). The nitrotyrosine and closed contact protons showed the strong chemical shifts to indicates the strong binding.





	H_A	H_B	H_F	H_H	H_J	H_K	H_L
δ (ppm)	3.064	2.959	4.576	3.765	7.829	6.881	7.327
δ* (ppm)	3.038	2.922	4.555	3.71	7.77	6.799	7.256
Δδ (ppm)	0.026	0.037	0.021	0.055	0.059	0.082	0.071

Figure 3. The NMR data showing the binding between the nitrotyrosine unit of the guest and the identified receptors.

e. <u>Task 4: Resynthesis of the identified synthetic receptor to separate the conformational isomers.</u> For the binding study, the identified receptors (trimers) were resynthesized using other simple guests. Since the trimers were composed of two conformational isomers, we tried to separate them using two different simple guests known to bind (Figure 4.1). The amplification was not clear in HPLC traces. However, the binding studies of each isomer using NMR studies indicates the separation of symmetric and asymmetric isomers. Since the symmetric isomer showed the almost identical NMR spectra to the previously obtained binding NMR data, it was assumed as the main contributor for the binding (Figure 4.2).

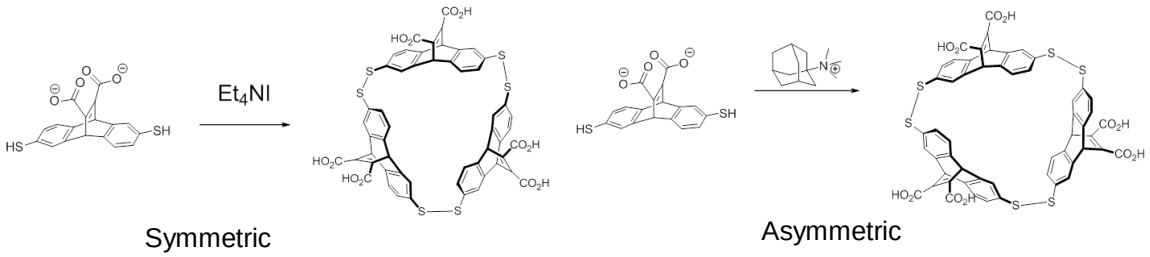
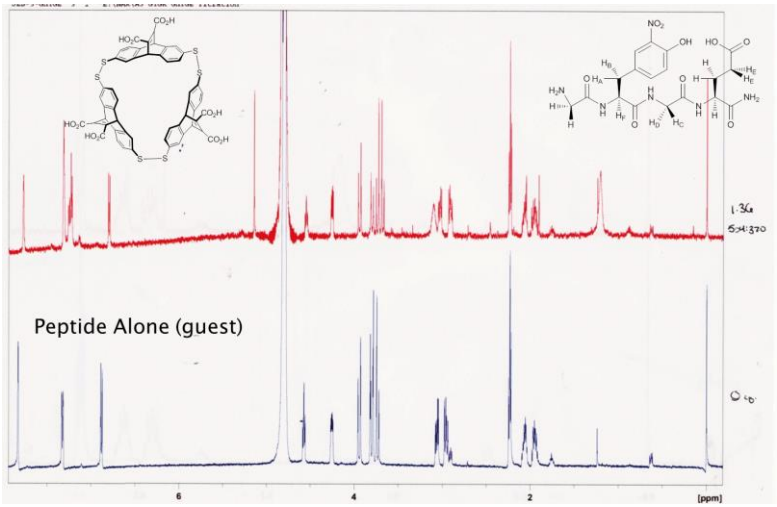
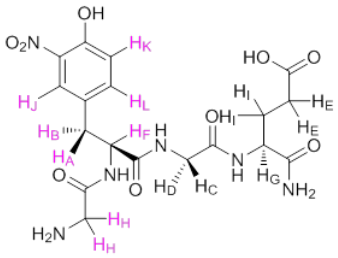


Figure 4.1. Two conformational isomer separation using two different guests.



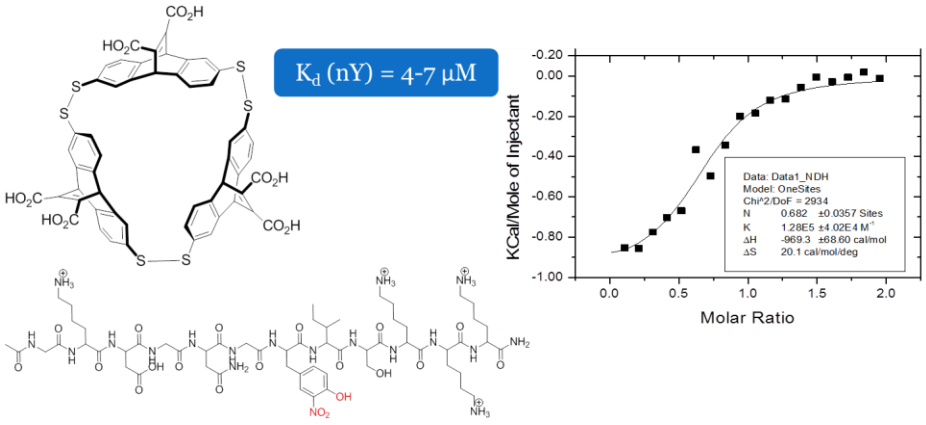


	H_A	H_B	H_F	H_H	H_J	H_K	H_L
δ (ppm)	3.064	2.959	4.576	3.765	7.829	6.881	7.327
δ* (ppm)	3.038	2.922	4.555	3.71	7.77	6.799	7.256
Δδ (ppm)	0.026	0.037	0.021	0.055	0.059	0.082	0.071

Figure 4.2. The NMR data indicating the symmetric isomer as the main contributor for the binding.

f. Task 5: <u>Determination of the binding constants between the target compounds and the identified synthetic receptors using anisotropy titration method and Isothermal Titration Calorimetry (ITC) analysis.</u> To determine the binding constant between the nitrotyrosine based peptide and the symmetric synthetic receptor, isothermal titration calorimetry (ITC) analysis and anisotropy titration methods were employed. With ITC, the K_d value was identified as 7 μM. The repeated experiments with anisotropy titration methods indicates the K_d values are 4 – 7 μM (Figure 5.1a). For the binding constant between the tyrosine based peptide and the symmetric synthetic receptor, anisotropy titration methods determined the K_d values are 48 μM (Figure 5.1b). It indicated the symmetric synthetic receptor has almost 8 times more selectivity for the nitrotyrosine based peptide over the tyrosine based peptide.

(a)



(b)

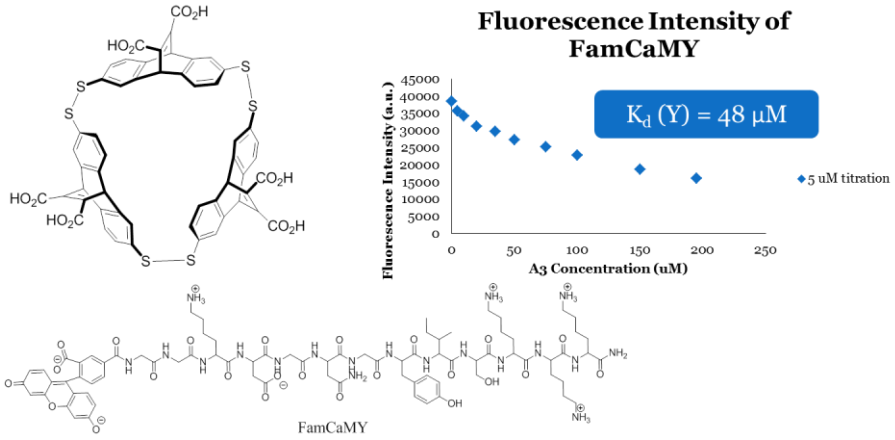


Figure 5.1. The binding constant determined by anisotropy titration methods between the symmetric receptor and (a) the nitrotyrosine peptide based guest, and (b) the tyrosine peptide based guest.

Additionally, the same methodologies were applied for the asymmetric receptors. With the ITC, the K_d value between the asymmetric receptors and the nitrotyrosine peptide based guest is 5.8 μM (Figure 5.2a). For the tyrosine peptide based guest, the corresponding K_d value is 19.8 μM (Figure 5.2b). With anisotropy titration methods, the K_d value between the asymmetric receptors and the tyrosine peptide based guest is 18.0 μM, which is similar to one obtained by the ITC. It indicated the symmetric synthetic receptor has almost 3 times more selectivity for the nitrotyrosine based peptide over the tyrosine based peptide.

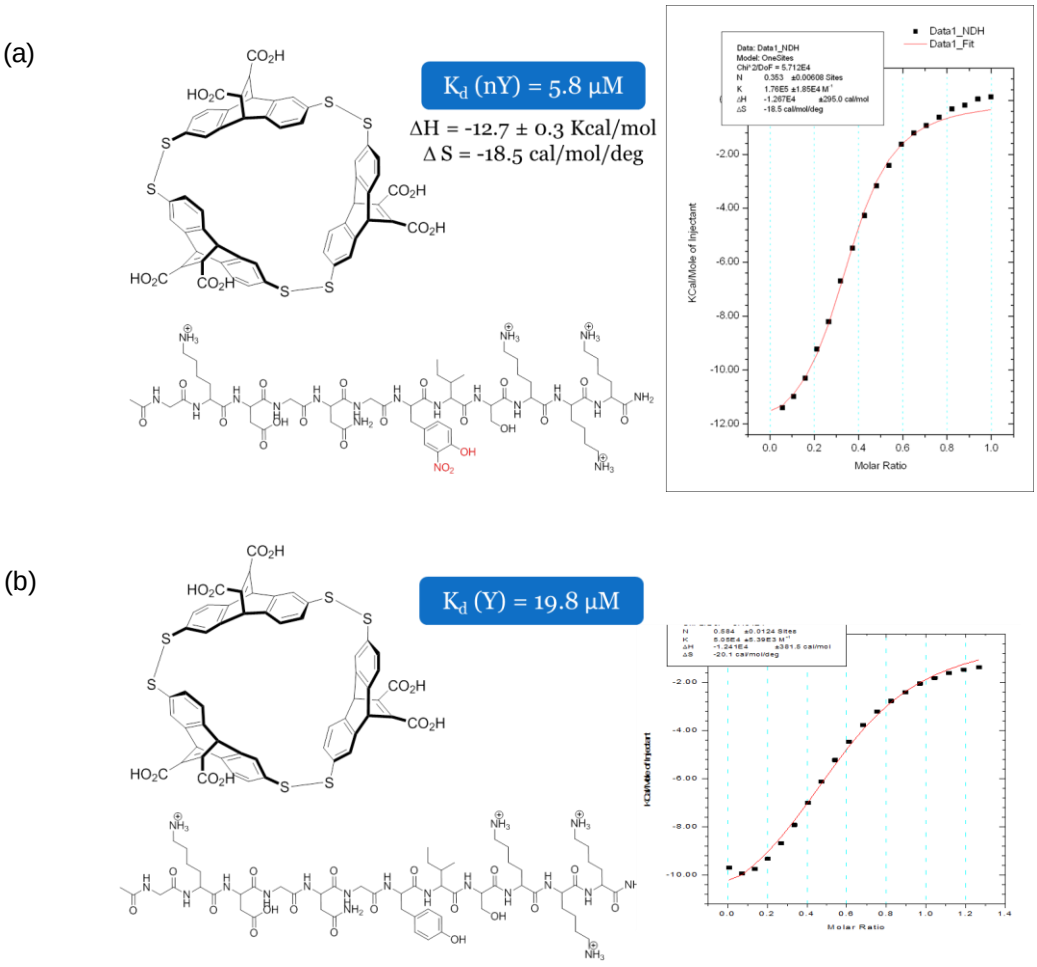


Figure 5.2. The binding constant determined by anisotropy titration methods between the asymmetric receptor and (a) the nitrotyrosine peptide based guest, and (b) the tyrosine peptide based guest.

g. <u>Task 6: Synthesis of thiol/thioester monomers for thioester exchange.</u> Using a peptide synthesizer, we generated a wide range of thiol/thioester monomers (Figure 6.1a).

These peptides have a cysteine unit as the thiol and a thioester in the C-terminus of the peptide. Other amino acids were varied. In addition, we synthesized monomers having hydroxyquinoline moiety as the N-terminal protecting group (Figure 6.1b). These monomers were used for the library screening for the binding. We also synthesized new peptide-based monomers to use for protein –protein interaction inhibition (target protein: MDM2) (Figure 6.1c) These monomers have L-tryptophan, L-leucine, L-phenylalanine, and the sequential L-proline and D-proline functions. These functions are known as the parts of three hydrophobic side chains in the tumor suppressor protein p53 and make direct contacts deep in the MDM2 cleft (Figure 6.1d).

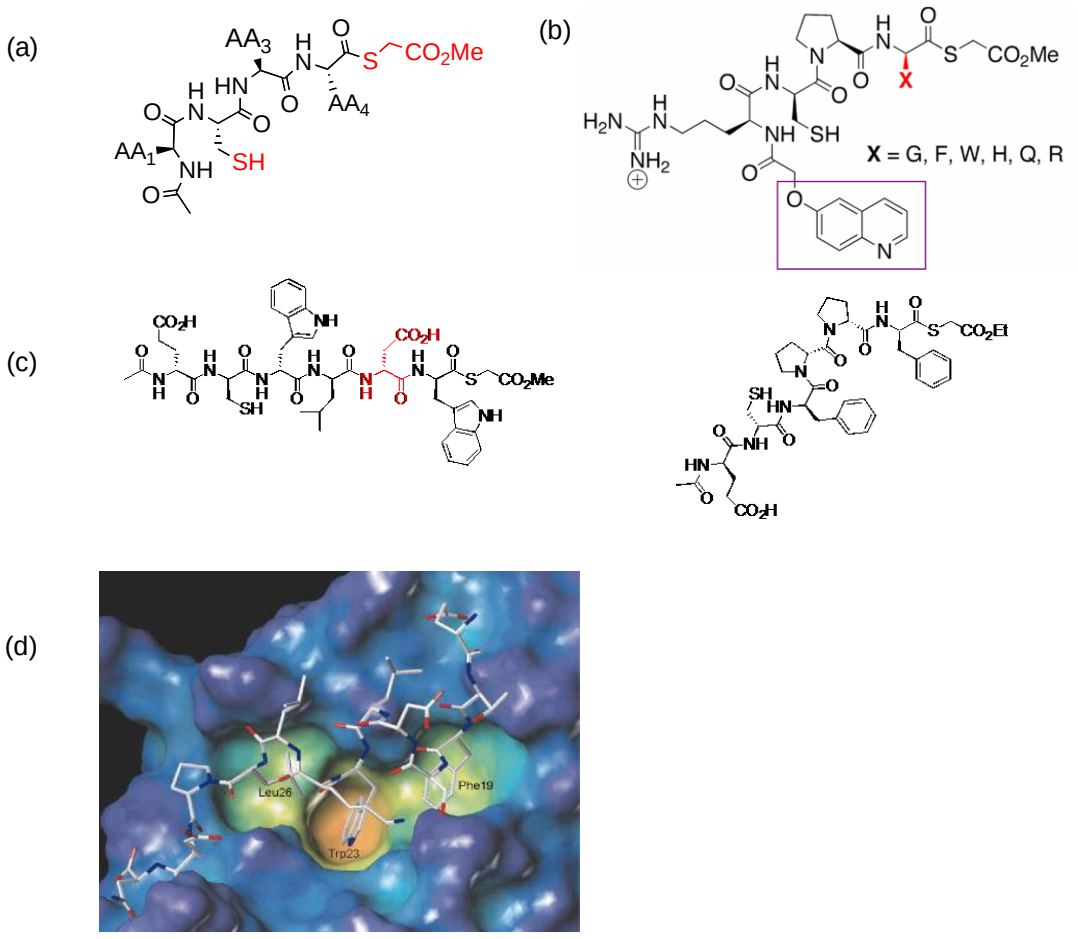


Figure 6.1. (a) Peptide-based monomers for thioester exchange. (b) N-terminal protected peptide-based monomers. (c) Thiol/thioester monomers for protein –protein interaction inhibition (target protein: MDM2). (d) Hot-spot on tumor suppressor protein p53/MDM2 interface.

Additionally, We synthesized new thiocoraline analog peptide-based monomers (Figure 6.2a) since thiocoraline is known to bind to duplex in cancer cells to active against human cancer cell lines (Figure 6.2b).

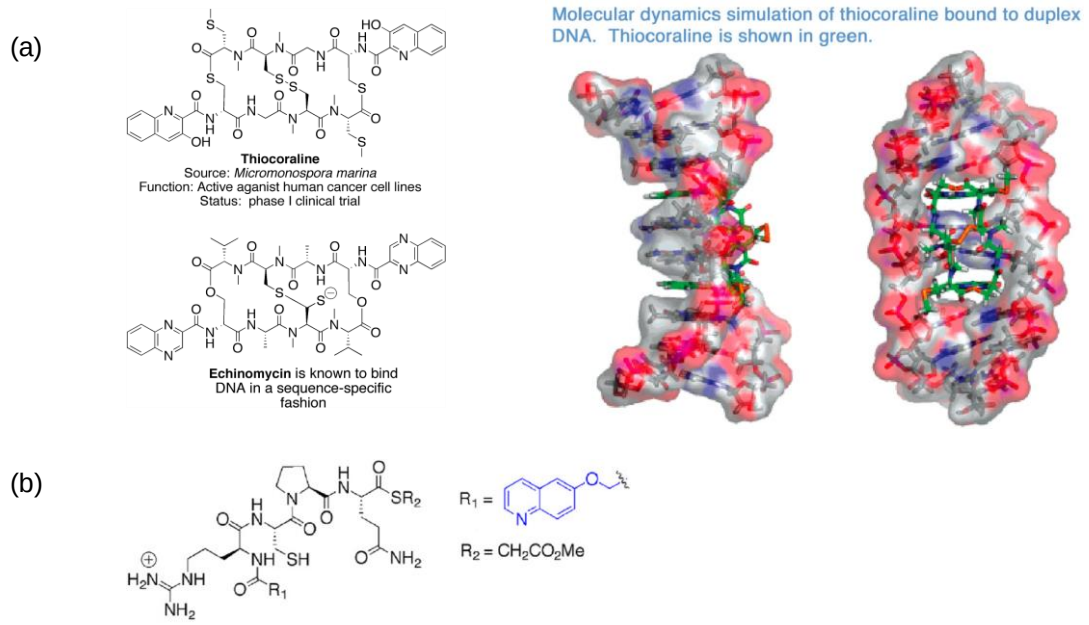
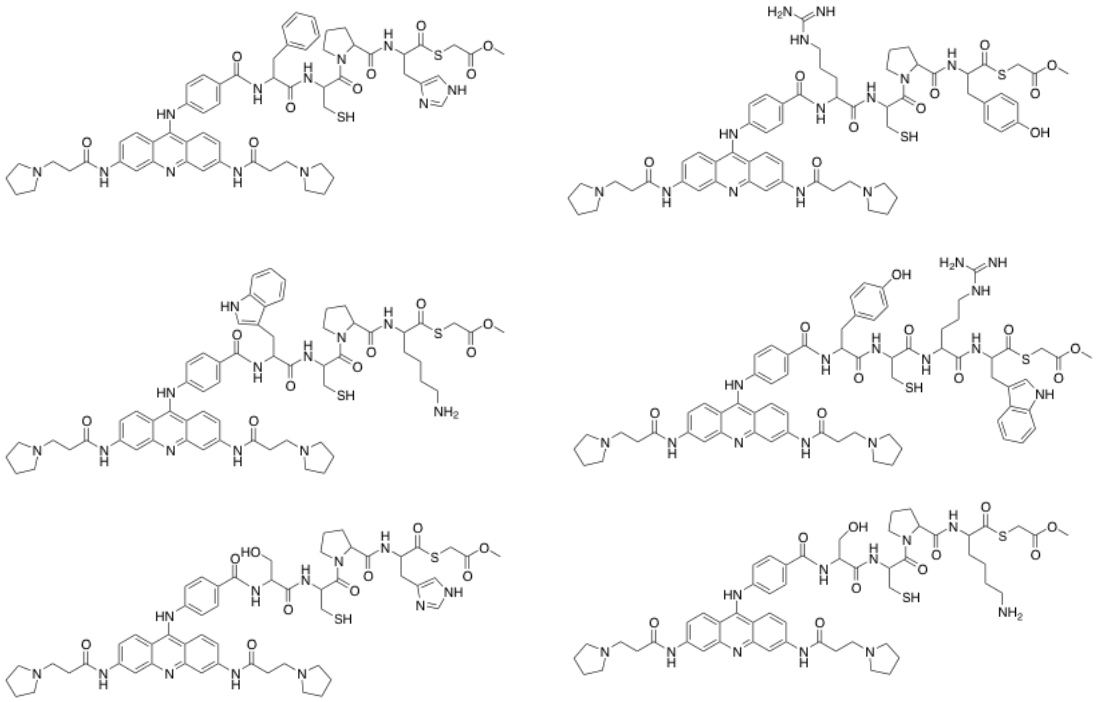


Figure 6.2. (a) Thiocoraline and simulation of thiocoraline bound to duplex DNA. (b) Newly developed thiocoraline analog peptide-based monomers.

We also synthesized thiol/thioester monomers (Figure 6.3) forming acridine-cyclic pepetide conjugates through thol/thioester exchange. These conjugates were expected to bind to and stabilize telomeric G-quadruplexes and lead to the inhibition of telomerase, playing a key role in the cancer cell development.



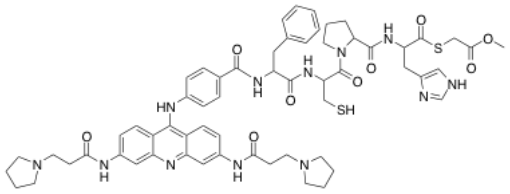
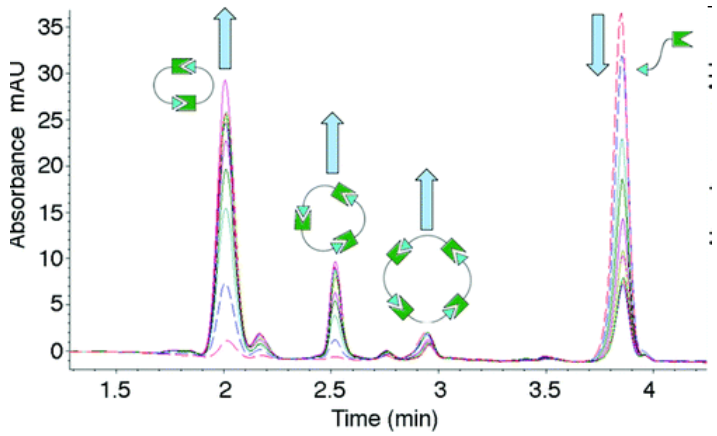


Figure 6.3. Newly developed thiol/thioester monomers forming acridine-cyclic pepetide conjugates through thol/thioester exchange.

h. Task 7: Evaluation of kinetics of thioester exchange and its structure-dependence. We found that the thiol/thioester monomers formed a mixture of macrocycles with dimeric cyclic thiodepsipeptide as the major product through the thiol-thioester exchange. Based on HPLC analysis of the reaction mixture over time (Figure 7a), a simple two-step mechanism could be proposed (Figure 7b). Two monomers react to form a dimer followed by the ring closing intramolecular trans thioesterification reaction. We have found that the rate of thioester exchange generally is fast, with $t_{1/2}$ of two hours or less for most compounds at pH 6.75 (Table 7). The reactions are faster at pH 7 in most cases ($t_{1/2}$ < 30 min). We found that steric hindrance at the C-terminus decreases the reaction rate (**1**~**2**>**3**~**4**~**5**>**6**>**7**, in Table 7) and the positively charged amino acids at either C-terminus or N-terminus accelerates the reaction rate (**10**-**15** in Table 7).

(a)



(b)

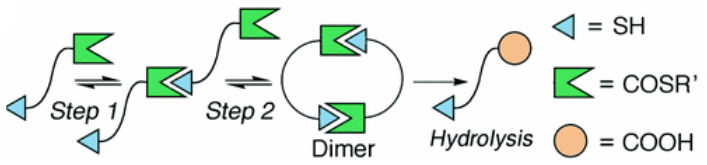


Figure 7. (a) Overlay of HPLC traces showing the consumption of monomers and the appearance of macrocycles over time. (b) Sequence of steps toward dimeric cyclic thiodepsipeptide.

compd	sequence	$t_{1/2}$	product
1	Ac-ECHyp**G**-COSR'	~12 min	dimer
2	Ac-ECP**H**-COSR'	~12 min	dimer, trimer
3	Ac-ECHyp**F**-COSR'	~1.5 h	dimer
4	Ac-EC**P**F-COSR'	~1.5 h	dimer
5	Ac-ECHyp**Q**-COSR'	~1.5 h	dimer, trimer, tetramer
6	Ac-ECHyp**E**-COSR'	~5.5 h	dimer
7	Ac-ECHyp**V**-COSR'	ND[a]	oligodimer
8	Ac-EC**W**E-COSR'	~3.5 h	dimer
9	Ac-EC(**D-P**)Q-COSR'	~10 min	dimer, trimer
10	Ac-**K**CP**R**-COSR'	~15 min	dimer
11	Ac-**R**CP**K**-COSR'	~15 min	dimer
12	Ac-**K**CP**K**-COSR'	~15 min	dimer
13	Ac-KCP**Q**-COSR'	~20 min	dimer, trimer
14	Ac-KC**W**R-COSR'	~15 min	dimer
15	Ac-KCP**V**-COSR'	~10 h	dimer, trimer

[a] Not determined. Monomers were hydrolyzed before complete reaction.

Table 7. Monomers, half-life of reactions, and conversions of **1**-**15** (100 mM NH_4Ac buffer, pH 6.75, 25 ^oC).

i. Task 8: <u>Screening for anion binding with thiol/thioester libraries.</u> Using developed peptide-based monomers, we have identified a dimeric compound (Figure 8a) in the thiol/thioester libraries that binds duplex DNA. Using the linear analog (Figure 8c), the binding affinity of the resulted cyclic receptor to duplex DNA was compared with that of the linear analog. The cyclic peptide had three fold higher affinity (K_d = 15 uM) for duplex DNA than its linear analog (K_d = 50 uM).

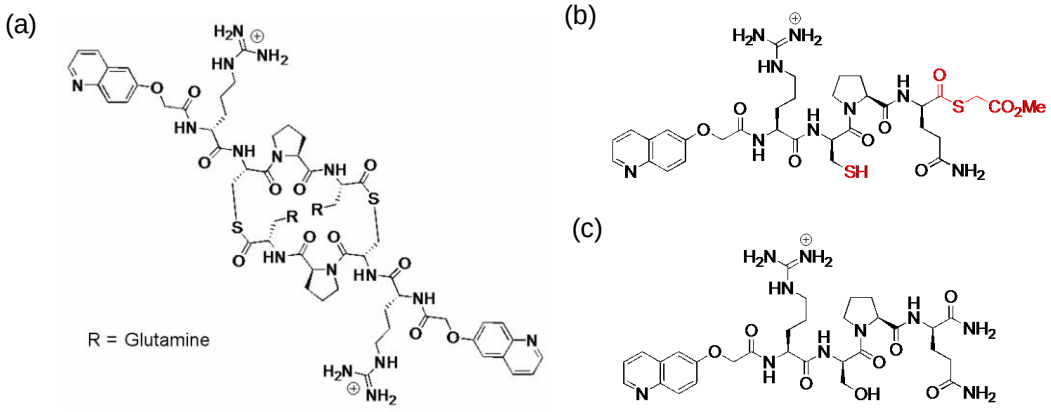


Figure 8. (a) An Identified dimeric compound showing binding affinity to duplex DNA. (b) Monomer generating a cyclic dimeric compound with binding affinity to duplex DNA and (c) its linear analog.

j. Task 9: <u>Development of DCL anaylsis methods using size exclusion chromatography.</u> We set up the DCL analysis methods using size exclusion chromatography with gels (Figure 9). In this chromatography, small particles can enter gel and traverse longer,

while large particles cannot enter gel and excluded and elute sooner. This technique was applied to the elusion of cyclic peptides, MDM2, and Bovine Serum.

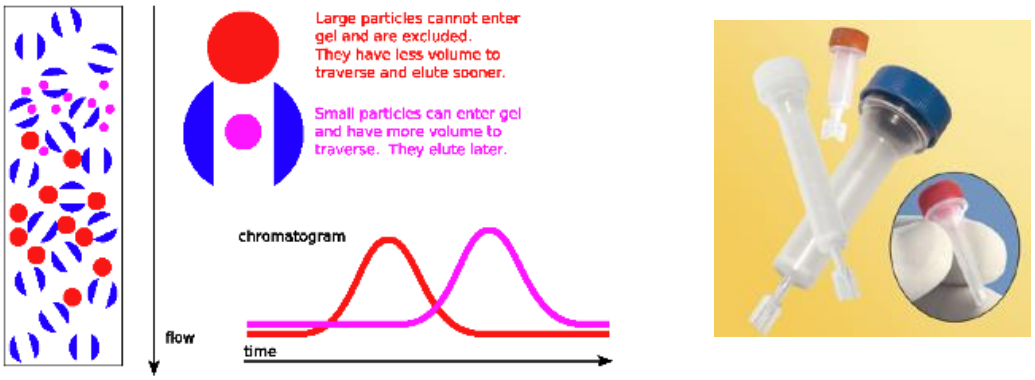


Figure 9. Size exclusion chromatography.

3. PLANNED ACTIVITIES FOR NEXT QUARTER

None

4. Performance Issues/Impacts

None

5. Schedule Issues/impacts

None

6. Cost Issues/Impacts

None